

AMENDMENT TO CLAIMS

1. (Currently Amended) A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:
 - (i) providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site that is cleavable by human Granzyme B protease, and wherein the recognition site comprises an amino acid sequence of the general formula

P4 P3 P2 P1 ↓ (SEQ ID NO: 59)

wherein

P4 is amino acid I or V,

P3 is amino acid E, Q or M,

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is said Granzyme B protease cleavage site, and
 - (c) ~~a the polypeptide~~ the polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest, and
 - (ii) cleaving the fusion protein with Granzyme B protease at said cleavage site to yield said polypeptide of interest in authentic form.
2. (Cancelled)
3. (Cancelled)
4. (Previously Presented) The method according to claim 1 wherein the N-terminus of the polypeptide of interest is adjacent to the cleavage site and the penultimate amino acid at the N-terminus of the polypeptide of interest is glycine.
5. (Cancelled)

6. (Previously Presented) The method according to claim 1, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.
7. (Cancelled)
8. (Previously Presented) The method according to claim 6, wherein the enzyme is Granzyme B.
9. (Previously Presented) The method according to claim 1, wherein the fusion partner is an affinity-tag.
10. (Previously Presented) The method according to claim 9, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.
11. (Previously Presented) The method according to claim 1, wherein the fusion protein is cleaved with a Granzyme B protease selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.
12. (Withdrawn) A method according to claim 11, wherein the Granzyme B protease is a human Granzyme B protease variant as shown in SEQ ID NO 57, wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.
13. (Previously Presented) The method according to claim 1, wherein the Granzyme B protease is in an immobilised form.
14. (Previously Presented) The method according to claim 13, wherein the Granzyme B protease is immobilised via the C-terminus.

15. (Previously Presented) The method according to claim 13, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.
16. (Previously Presented) The method according to claim 10, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with said Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).
17. (Previously Presented) The method according to claim 16, wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.
18. (Withdrawn) A fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, and (c) a polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest.
19. (Withdrawn) A fusion protein according to claim 18, wherein the Granzyme B protease recognition site comprises an amino acid sequence of the general formula:

P4 P3 P2 P1 ↓

wherein

P4 is amino acid I or V,

P3 is amino acid E, Q or M,

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is said Granzyme B protease cleavage site.

20. (Withdrawn) A fusion protein according to claim 18, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓, IEAD↓, IEPD↓, IETD↓, IQAD↓, ISAD↓, ISSD↓, ITPD↓, VAPD↓, VATD↓, VCTD↓, VDPD↓, VDSD↓, VEKD↓, VEQD↓, VGPD↓, VEID↓,

VRPD↓, VTPD↓, LEED↓, LEID↓, LGND↓, LGPD↓, and AQPD↓, and wherein ↓ is said Granzyme B protease cleavage site.

21. (Withdrawn) A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general formula P4 P3 P2 P1↓P1'P2', wherein P1' is X where X denotes any amino acid, P2' is G, and wherein P1' and P2' is a part of the polypeptide of interest.
22. (Withdrawn) A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids P1', P2' P3' and P4' resulting in the general formula P4 P3 P2 P1↓P1'P2'P3'P4', wherein P4' is D or E, and wherein P1', P2', P3' and P4' is a part of the polypeptide of interest.
23. (Withdrawn) A fusion protein according to claim 18, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.
24. (Withdrawn) A fusion protein according to claim 23, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and inteferon.
25. (Withdrawn) A fusion protein according to claim 23, wherein the enzyme is Granzyme B.
26. (Withdrawn) A fusion protein according to claim 25, wherein Granzyme B comprises a C-terminal polyhistidine-tag.
27. (Withdrawn) A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 (SEQ ID NO 2) and pro-IEAD-GrB-H6 (SEQ ID NO 3).
28. (Withdrawn) A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 C228A (SEQ ID NO 5), pro-IEPD-GrB-H6 C228T

(SEQ ID NO 6), pro-IEPD-GrB-H6 C228V (SEQ ID NO 7), and pro-IEPD-GrB-H6 C228F (SEQ ID NO 8).

29. (Withdrawn) A fusion protein according to claim 25, wherein the enzyme Granzyme B is a human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.
30. (Withdrawn) A fusion protein according to claim 25, wherein the human Granzyme B protease variant is as shown in SEQ ID NO 57.
31. (Withdrawn) A fusion protein according to claim 18, wherein the fusion partner is an affinity-tag.
32. (Withdrawn) A fusion protein according to claim 31, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.
33. (Withdrawn) A human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.
34. (Withdrawn) A human Granzyme B protease variant according to claim 33, as shown in SEQ ID NO 57.
35. (Withdrawn) A method of cleaving a fusion protein comprising contacting said fusion protein with the human Granzyme B protease variant according to claim 33.
36. (Withdrawn) An isolated nucleic acid sequence encoding the fusion protein according to claim 19 or the human Granzyme B protease variant according to claim 33.
37. (Withdrawn) A recombinant vector comprising the isolated nucleic acid sequence according to claim 36.

38. (Withdrawn) A host cell transformed with a vector according to claim 37.
39. (Withdrawn) A method for the production of a fusion protein according to claim 18 or a human Granzyme B protease variant according to claim 33, comprising the steps of:
- (i) providing a recombinant vector comprising the isolated nucleic acid sequence according to claim 36 operatively linked to a promotor,
 - (ii) transforming a host cell with said recombinant vector,
 - (iii) culturing said host cell under conditions to express said fusion protein or human Granzyme B protease variant, and
 - (iv) optionally isolating said fusion protein or human Granzyme B protease variant.
40. (Currently Amended) A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:
- (i) providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site that is cleavable by human Granzyme B, wherein the recognition site comprises an amino acid sequence selected from the group consisting of ICPD↓ (SEQ ID NO: 61), IEAD↓ (SEQ ID NO: 62), IEPD↓ (SEQ ID NO: 63), IETD↓ (SEQ ID NO: 64), IQAD↓ (SEQ ID NO: 65), ISAD↓ (SEQ ID NO: 66), ISSD↓ (SEQ ID NO: 67), ITPD↓ (SEQ ID NO: 68), VAPD↓ (SEQ ID NO: 69), VATD↓ (SEQ ID NO: 70), VCTD↓ (SEQ ID NO: 71), VDPD↓ (SEQ ID NO: 72), VDSD↓ (SEQ ID NO: 73), VEKD↓ (SEQ ID NO: 74), VEQD↓ (SEQ ID NO: 75), VGPD↓ (SEQ ID NO: 76), VEID↓ (SEQ ID NO: 77), VRPD↓ (SEQ ID NO: 78), VTPD↓ (SEQ ID NO: 79), LEED↓ (SEQ ID NO: 80), LEID↓ (SEQ ID NO: 81), LGND↓ (SEQ ID NO: 82), LGPD↓ (SEQ ID NO: 83), and AQPD↓ (SEQ ID NO: 84), and

wherein ↓ is said Granzyme B protease cleavage site, and the polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest, and

- (ii) cleaving the fusion protein with Granzyme B protease at said cleavage site to yield said polypeptide of interest in authentic form.
- 41. (Previously Presented) The method according to claim 40, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.
 - 42. (Cancelled)
 - 43. (Previously Presented) The method according to claim 41, wherein the enzyme is Granzyme B.
 - 44. (Previously Presented) The method according to claim 40, wherein the fusion partner is an affinity-tag.
 - 45. (Previously Presented) The method according to claim 44, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.
 - 46. (Previously Presented) The method according to claim 40, wherein the fusion protein is cleaved with a Granzyme B protease selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.
 - 47. (Previously Presented) The method according to claim 40, wherein the Granzyme B protease is in an immobilised form.
 - 48. (Previously Presented) The method according to claim 47, wherein the Granzyme B protease is immobilised via the C-terminus.

49. (Previously Presented) The method according to claim 47, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.
50. (Previously Presented) The method according to claim 44, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with said Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).
51. (Previously Presented) The method according to claim 50, wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.